Gene technology

Gene technology is the manipulation of genes for the benefit of people, in areas such as agriculture, food production and medicine. The tools and techniques of the genetic engineer are shown in Table 4.

Table 4 The tools and techniques of gene technology

Tool/technique	Purpose
Restriction enzymes	To cut DNA at specific points, making small fragments
DNA ligase	To join DNA fragments together
Vectors	To carry DNA into cells and ensure replication
Plasmids	Commonly used vectors
Genetic markers	To identify cells that have been transformed
PCR	To amplify very small samples of DNA
cDNA	To make a DNA copy of mRNA
DNA probes	To identify and label a piece of DNA containing a certain sequence
DNA sequencing	To read the base sequence of a length of DNA

Links Various DNA technologies, including restriction endonuclease enzymes, genetic markers, the polymerase chain reaction (PCR), DNA probes and DNA profiling (fingerprinting), are covered in the AS Unit 1 guide (pp. 38–43) in this series, to which you should refer.

Genetically modified organisms

Genetically modified organisms (GMOs) are also called genetically engineered organisms. Their production involves gene transfer, which is the transfer of a gene from a donor organism to a recipient organism.

Genetically engineered microorganisms (GEMs)

There are a number of stages in manipulating genes to produce genetically modified organisms and, specifically, genetically engineered microorganisms (GEMs):

- obtaining the required gene
- inserting the gene into a vector
- inserting the vector into a host cell
- identification of the host cells that have taken up the gene
- cloning the gene in the modified host cells

Obtaining the required gene

There are two main methods used to obtain a gene.

1 **Restriction endonuclease enzymes are used to cut the gene out of chromosomal DNA** (Figure 20):

- The chromosomal DNA is cut into fragments using an appropriate restriction endonuclease. The enzyme is 'appropriate' when it cuts either side of the gene.
- The DNA fragment containing the required gene is identified using a gene probe.
- A restriction endonuclease that cuts in a staggered fashion to produce 'sticky ends' is most useful. If the vector is opened with the same restriction enzyme, then the exposed bases of both are complementary and so will more readily attach through base pairing.

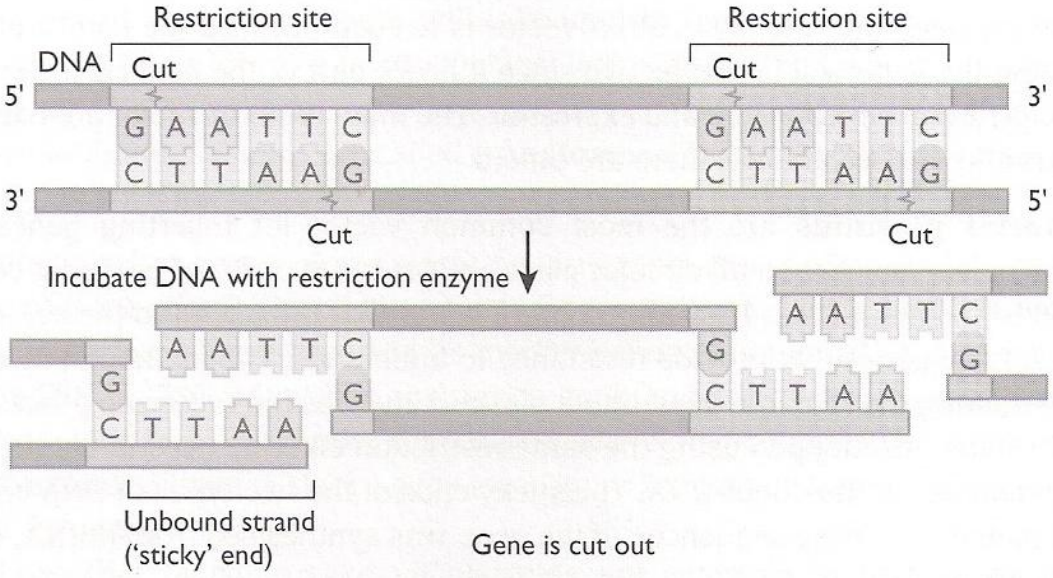

Figure 20 Use of restriction endonuclease enzymes to cut the gene out of chromosomal DNA

2 **Reverse transcriptase is used to produce DNA from mRNA** (Figure 21):

- Messenger RNA is obtained from cells where the gene concerned is actively synthesising protein (such cells possess many copies of the mRNA).

- The enzyme reverse transcriptase uses the mRNA as a template to produce a complementary single strand of DNA (complementary or cDNA) from free DNA nucleotides.
- Double-stranded DNA is made from the cDNA using the enzyme DNA polymerase.

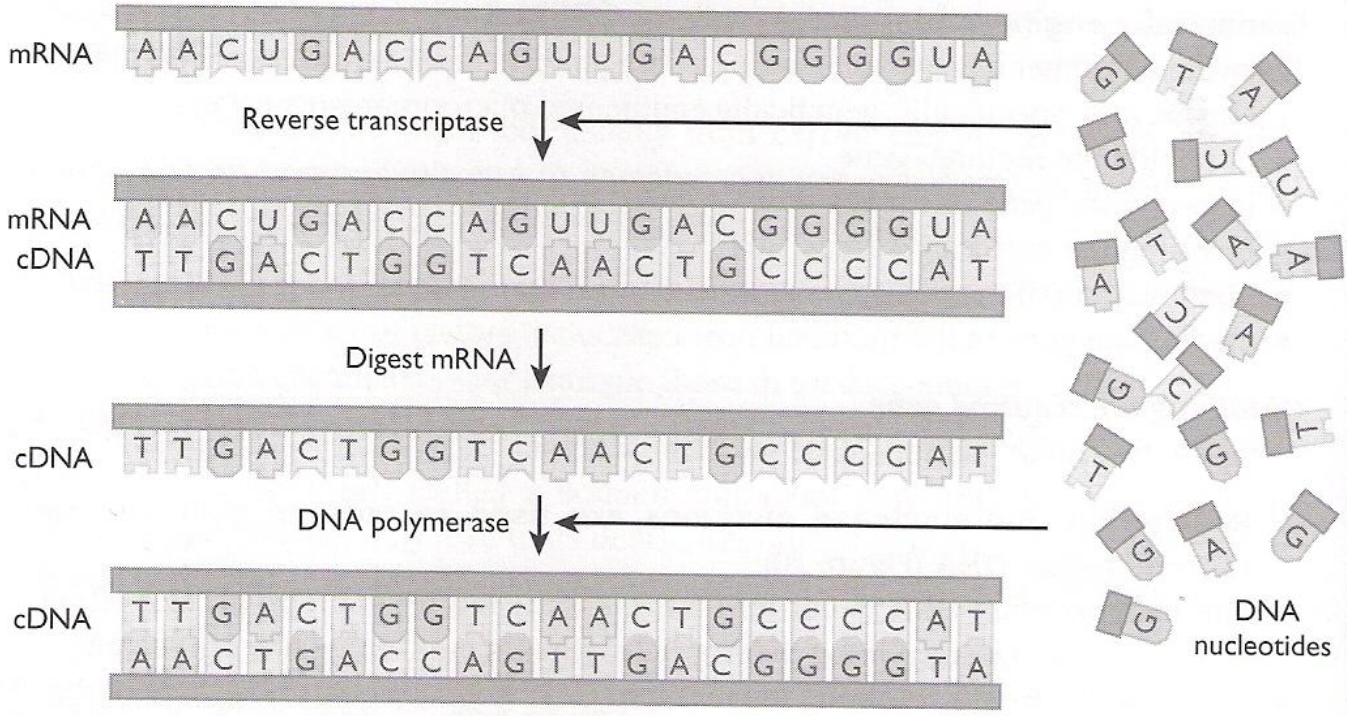

Figure 21 Use of reverse transcriptase to produce DNA from messenger RNA

Inserting the gene into a vector

Once the gene has been obtained it is inserted into a vector, which is a delivery tool to carry the gene into the host cell. A vector is needed because the length of DNA containing the gene will be ineffective since it is not part of the host cell's genome. In a vector, it can be replicated and expressed. The main types of vector are bacterial plasmids and viruses, though there are others.

1 Bacterial plasmids are the most common vector for inserting genes into bacterial cells. They are small circular pieces of DNA that occur in bacterial cells in addition to the main DNA. They may contain genes that are useful to the bacterium — for example genes that provide resistance to antibiotics. The method of inserting DNA containing the required gene into a plasmid involves the following processes:

- The plasmid is cut open using the same restriction enzyme used to cut the DNA fragment out of the donor DNA. The sticky ends of the two types of DNA contain complementary base sequences (if the gene was synthesised from mRNA, sticky ends are added).
- The plasmid DNA and gene DNA anneal (join). Hydrogen bonds form readily between the complementary bases of the sticky ends and **DNA ligase** catalyses the formation of covalent bonds (phosphodiester bonds) between the sugar-phosphate backbones of the plasmid DNA and the gene DNA. The gene is said to be **spliced** into the plasmid.

Any DNA that has 'foreign DNA' inserted into it is called **recombinant DNA**, so the plasmid is now a **recombinant plasmid**.

This process is shown in Figure 22.

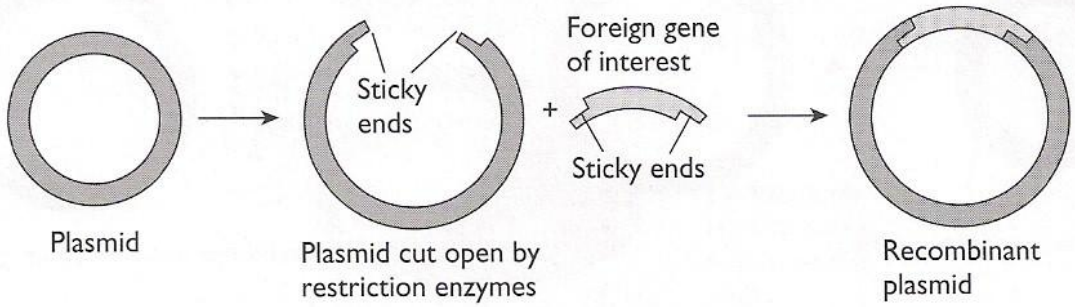

Figure 22 Transferring a gene into a plasmid

2 Viruses are adapted to insert their genetic material into a host cell — for example, a bacteriophage 'injects' its DNA into a bacterium. A bacteriophage that has a DNA fragment spliced into its DNA will transfer that recombinant DNA into the bacterial cell.

Inserting the vector into a host cell

Bacterial cells do take up plasmids — they are used naturally for the exchange of genes between bacterial cells. However, they do so more readily when induced. Cells are incubated with calcium ions and treated with a heat shock (temperature rise from 0°C to 40°C), which makes the cell wall permeable to plasmids. Bacteriophages are an effective way of delivering large genes into bacterial cells.

Identification of the host cells that have taken up the gene

Only a few of the bacterial cells will take up a recombinant plasmid — the rate of take-up may be as low as 1 in 10000. Most will either fail to take up a plasmid at all or will take up an original, non-transformed plasmid. Bacteria that have taken up the recombinant plasmids are called **transformed bacteria**. The two main ways of identifying such bacteria are marker genes and gene probes.

1 Using marker genes. Some plasmids carry genes that confer antibiotic resistance to the bacteria. The R-plasmid has genes for resistance to two antibiotics, ampicillin and tetracycline. These are used as marker genes. The restriction enzyme cuts in the middle of the tetracycline resistance gene and the 'desired' gene is inserted. The transformed plasmid now contains an active ampicillin resistance gene but an inactive tetracycline resistance gene (see Figure 23). Bacteria are detected according to the following criteria:

- Those that failed to take up plasmids are sensitive to both ampicillin and tetracycline.
- Those that take up the original plasmid are resistant to both ampicillin and tetracycline.
- Those that take up the recombinant plasmids are resistant to ampicillin but not to tetracycline.

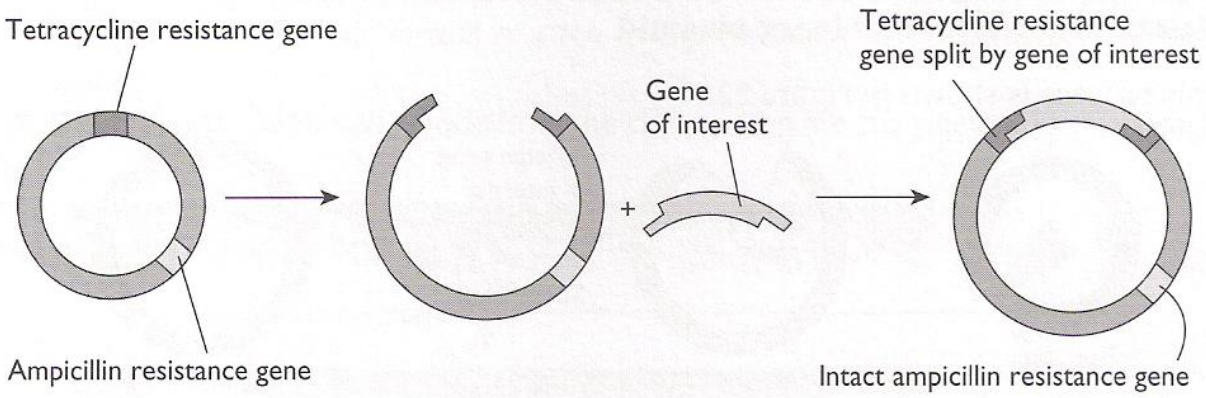

Figure 23 Inserting a gene into a plasmid with antibiotic resistant marker genes

The bacteria are cultured on agar plates. Each bacterium multiplies to form a colony. The colonies on the agar plate are then replica-plated onto a plate containing ampicillin and a plate containing tetracycline. Replica-plating involves 'blotting' the original plate with a pad and then pressing this against the surface of a fresh plate so that a few cells from each colony are transferred. The bacteria that survive on the ampicillin plate *only* are the transformed bacteria.

2 Using gene probes. A DNA or gene probe is a length of DNA — a 20-base sequence is sufficient — that is complementary to part of the gene being sought. Gene probes are produced so that each can base-pair with a complementary section of the DNA that makes up the gene. The gene probe and, therefore, the gene to which it can attach ('hybridise') can be identified if the probe is radioactively labelled. Figure 24 summarises the technique. Fluorescently labelled probes can also be used. They are detected using ultraviolet light.

Cloning the gene in the modified host cells

When the transformed bacteria are cultured their DNA replicates and the cells divide. As a result, many copies or **clones** of the desired gene are produced. These clones can be used to produce useful protein. This gene cloning technique can also be used to produce many copies of a healthy human gene, potentially for use in gene therapy.

Genetically modified plants

Foreign genes are inserted into plant cells using a range of methods.

- The most common method of transferring genes into plant cells is to use the common soil bacterium *Agrobacterium tumefaciens*, which readily invades damaged plant tissue and causes tumour-like growths. On entering the damaged tissue the bacterium's **tumour-inducing (Ti) plasmid** is transferred into a plant cell. For gene transfer, the desired gene is spliced into the plasmid, which is readily taken up by the plant cell provided that its cellulose wall has been removed by treatment with the enzyme cellulase.
- Minute pellets that are covered with DNA carrying the desired gene are shot through the cellulose walls into plant cells using a particle gun.

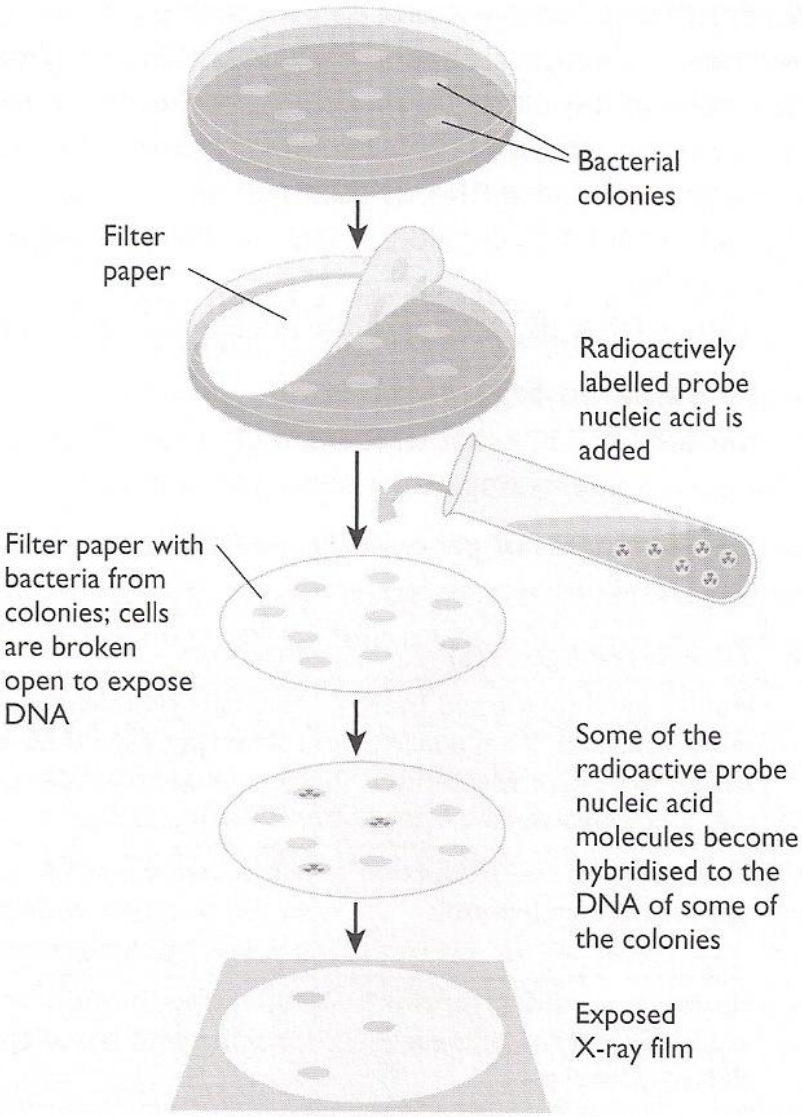

Figure 24 Using a radioactive gene probe to identify transformed bacteria with the desired gene

- Plant viruses are sometimes used. They infect cells by inserting their nucleic acid and, therefore, new genes can be transferred into the cell.

A gene for antibiotic resistance is inserted along with the desired gene. This is so that treatment with the antibiotic will kill the non-transformed cells, leaving only those that have been transformed. Transformed plant cells are then tissue-cultured and entire genetically modified plants are grown from this tissue.

Genetically modified animals

Several techniques can be used to artificially introduce genes into animal cells:

- A common method of introducing DNA into an animal cell is by **electroporation**, a technique in which the cell membrane is temporarily disrupted through treatment with a high-voltage shock.
- Another approach is to coat DNA in minute artificial lipid vesicles, called **liposomes**, which may adhere to the cell-surface membrane and pass the DNA into the cell in a similar way to endocytosis.

- **Viruses** (adenoviruses and retroviruses) can be used to insert genes into animal cells. Adenoviruses are human viruses that can cause respiratory diseases. Therefore, they have to be altered genetically so that the host cells are not destroyed. Adenoviruses are particularly useful for delivering genes to patients in gene therapy. Retroviruses have RNA as their genetic material. When their RNA is delivered into a host cell it is copied to DNA and the DNA is incorporated into the host's chromosome.
- It is possible to **inject DNA directly** into the nucleus of a fertilised egg.

Uses of genetically modified organisms

There are a huge number of GMOs that have been engineered for use in medicine, agriculture and industry. Some examples are shown in Table 5.

Table 5 Some uses of genetically modified organisms

Genetically modified organism	Transferred gene(s)	Purpose
Bacteria (*Escherichia coli*)	Human gene (produced from mRNA isolated from pancreatic tissue) for the production of the hormone insulin	Insulin is produced for use by people who cannot produce their own (suffer from type I diabetes mellitus)
Bacteria (*E. coli*) and fungi (*Aspergillus niger*)	Cow gene for the production of the enzyme chymosin	Chymosin is a coagulating enzyme used in the commercial production of hard cheese; most is genetically engineered
Yeast (*Saccharomyces cerevisiae*)	Human gene coding for the production of the protein alpha-1-antitrypsin	Alpha-1-antitrypsin is used to treat patients with hereditary emphysema
Soya (*Glycine max*)	A gene that makes the plants resistant to a specific herbicide (e.g. glyphosate)	Spraying with the herbicide kills competing weeds and so the productivity of the resistant crop plant is increased
Maize (*Zea mays*)	The *Bt* gene from the bacterium *Bacillus thuringiensis*	The gene produces a protein that is toxic to some insects (mainly caterpillars) so reducing the use of insecticides
Rice plants (*Oryza sativa*)	Two genes, each of which produces an enzyme involved in the metabolic production of beta-carotene (vitamin A)	Produces 'golden rice' with high levels of vitamin A used to supplement the diet in developing countries where people suffer permanent blindness from a lack of vitamin A
Cows	Human gene *LALBA* that codes for the synthesis of the protein alpha-lactalbumin	Produces human protein-enriched milk, more nutritionally balanced than natural cow's milk (for babies or the elderly with special nutritional needs)
Sheep (ewes)	Human gene for the blood-clotting protein Factor VIII	Factor VIII is extracted from the milk of transgenic ewes and used as a treatment for haemophilia
Chickens	Human gene for the production of the antibody mi-R24	The mi-R24 antibody is extracted from eggs and used in the treatment of the skin cancer malignant melanoma

Tip You do not have to learn a lot of examples of gene transfer but you should be familiar with some. Any example of a GMO in a question is likely to be unfamiliar. This is deliberate. You are being asked to apply your understanding of principles and procedures.

Gene therapy

Gene therapy is the treatment of a genetic disease by introducing the functional allele of the gene into the affected cells. Although attempts have been made to treat several different diseases (e.g. sickle-cell anaemia and muscular dystrophy) using gene therapy there are still major problems to be solved before treatments become sufficiently successful to be used widely.

Gene therapy for cystic fibrosis

Cystic fibrosis is a genetic disorder resulting from the mutation of a gene that codes for a carrier protein called CFTR. This protein lies in the cell-surface membrane of cells in many parts of the body, including the lungs, pancreas and reproductive organs. It transports chloride out of the cells. Water follows by osmosis. When the CFTR protein is not working, this does not happen. There is therefore much less water on the outer surface than there should be. The mucus that is produced in these areas therefore does not mix with water in the usual way. The mucus is thick and sticky. As a result:

- the abnormally thick mucus collects in the lungs, interfering with gas exchange and increasing the chance of bacterial infections
- the pancreatic duct may become blocked with sticky mucus, interfering with digestion in the small intestine
- reproductive passages (e.g. the oviducts) may become blocked, making a person sterile

Attempts have been made to treat cystic fibrosis by introducing the normal CFTR allele into the affected cells. Two methods of gene transfer have been trialled:

- inserting the normal allele into a modified adenovirus and then allowing the virus to infect cells in the person's respiratory passages — the virus enters the cells and so introduces the gene
- inserting the normal allele into liposomes and spraying these as an aerosol into the person's respiratory passages

In each case, there was limited success in that some of the cells lining the respiratory passages did take up the gene. However, there were also problems with these trials, including the following:

- Only a few cells took up the normal allele, so only those cells produced normal mucus.
- Cells in the surfaces of the respiratory passages do not live for long, so treatment would need to be repeated every few weeks.
- When using the adenovirus as a vector, some people developed serious lung infections.

- It was only possible for cells in the respiratory passages to take up the normal allele, not cells in the pancreas or reproductive organs.

Gene therapy for SCID

Gene therapy has also been used to treat **severe combined immunodeficiency** (SCID), which is a rare autosomal recessive disorder. The first successful treatment of a child with SCID was in 1990. T-cells were removed, given the normal allele using a retrovirus as a vector, and the transformed T-cells were placed in her bone marrow. However, in later cases problems arose as patients developed a type of cancer called leukaemia.

Genome sequencing

The **genome** is the total genetic make-up of an organism, i.e. all of its DNA. The term originates from **gen**e and chromos**ome**. Genome is defined as the complete nucleotide (base) sequence in a haploid set of chromosomes of a eukaryotic cell. It is the complete set of genes, together with the non-coding DNA in between. Each genome contains the genetic coding needed to build and maintain that organism. **Genome sequencing** involves the laborious task of determining the order of nucleotides (bases) on each chromosome of a eukaryotic organism.

Genome sequencing projects have been undertaken for a range of organisms including the virus phage λ, the bacterium *Escherichia coli*, the plant *Arabidopsis thaliana*, the fruit fly *Drosphila melanogaster*, the mouse *Mus musculus* and humans. Those with smaller sequences were the first to be determined. The ultimate goal was the determination of the human genome sequence. Knowing the mouse genome is also important in that it is the model organism for much of the research into gene function.

Humans have 22 pairs of autosomes and two sex chromosomes. The **human genome project** worked on 24 separate chromosome sequences (22 autosomes and both X and Y chromosomes). The exact order of over 3 billion base pairs that make up these 24 chromosomes has been determined. Achieving this goal has helped to reveal an estimated 20 000 to 25 000 human genes in the total DNA. Many of these genes had been previously unknown. It has also revealed that less than 5% of the DNA represents the genes. There is a lot of non-coding DNA — approximately 95% of the genome.

Genes code for the synthesis of proteins. The non-coding DNA — most of it — has been referred to as 'junk' DNA. However, it seems likely that some of the non-coding regions (in and surrounding genes) contain signals that have not yet been recognised. Some DNA sequences might have other functions — suggestions include chromosomal replication, packaging the DNA into highly condensed chromatin and control of development. It seems that some of the non-coding DNA acts as genetic 'switches' that do not encode proteins but regulate when and where genes are expressed.

While a working draft of the human genome is available, researchers are now concentrating on adding the detail about human genes and the variations that

can exist. The **HapMap project** aims to produce a map of human allele variants. Although any two unrelated people have about 99.9% of their DNA sequences in common, the remaining 0.1% is important because it contains the genetic variants that influence how people differ (e.g. in their risk of disease or their response to drugs). Sites in the genome where the DNA sequences of individuals differ by a single base are called single nucleotide polymorphisms (SNPs). About 10 million SNPs exist in human populations (where the SNP allele has a frequency of at least 1%).

Some of the *advantages of genome sequencing projects* are summarised below:

- A knowledge of the base sequence of genes allows the primary structure (amino acid sequence) of proteins to be determined. The use of molecular modelling software then allows the secondary, tertiary and quaternary structure to be predicted.
- A knowledge of the base sequence differences between different alleles (SNPs) should allow a better understanding of human diseases with a genetic cause. This in turn should facilitate their management and control through:
  - **gene therapy**, e.g. to treat cystic fibrosis
  - **genetic screening**. Genetic screening is used to determine if a person is at risk of passing on a hereditary disorder. The technique involves the use of 'DNA chips' — ordered series of nucleotide sequences that act as DNA probes. These allow the identification of a particular base sequence (specific to the 'disease' allele) and so determine if an individual is a carrier of a genetic disorder.
- Knowing the base sequences of different alleles (SNPs) provides improved diagnostics to test for the presence of genes that increase susceptibility of an individual to, for example, cancer or heart disease.
- Knowing the base sequences of different alleles (SNPs) provides an understanding of why different individuals respond differently to the same drugs. This may allow the development of 'designer drugs' matched to an individual's genetic profile.
- A number of genome sequencing projects are working on pathogenic micro-organisms. This should allow a better understanding of how microorganisms might act as pathogens. This should facilitate protection from the diseases they cause.
- An understanding of genome sequences should allow biologists to work out the various molecular interactions that lead to the normal development of organisms.
- Genome sequencing of different species allows direct comparisons between species. This will provide information that should allow evolutionary relationships to be determined.

Gene knockout technology

A **gene knockout** is a genetically engineered organism that carries a gene that has been made inoperative. This allows the function of genes to be studied. Comparing how the knockout organism differs from individuals in which that particular gene has not been made inoperative provides information about what the gene does. The

role of the gene in protein production and its metabolic and physiological influence can be determined. For example, imagine a gene GP, which makes the protein GP that controls the metabolism of glucose — a knockout model GP would be expected to lack the protein GP and have a problem with glucose metabolism.

Mice are the laboratory species most closely related to humans in which the knockout technique can be performed easily. A **knockout mouse** has both alleles of a particular gene inactivated. Knockout mice allow researchers to determine the role of a particular gene by observing what happens, both metabolically and physiologically, when gene function is lost. **Knockout models** (mice in which a specific gene has been 'knocked out') have been produced for studying human genetic diseases such as cystic fibrosis and thalassaemia — diseases in which the normal gene is defective. Mouse models have also been used extensively to study genes that become defective through mutation and cause cancer. More than 10000 mouse genes (approximately half the genes in the mammalian genome) have been knocked out. The **knockout mouse project** aims to make knockout mice for all genes available for scientific and biomedical research.

Issues surrounding the use of gene technology

Some people find gene technology controversial. In such circumstances there is a need to be as fully informed as possible in order to evaluate and discuss the issues rationally. There are obvious potential *benefits* and, therefore, arguments in favour of its use. However, there are potential *risks* that may be cited in arguments against its use. Some examples are shown in Table 6.

In order to reduce risks from the use of genetically modified microorganisms a number of **safety precautions** have been devised:

- use of bacterial strains ill-adapted to the human physiology, for example:
  - strains that grow more slowly than normal wild-type intestinal bacteria so that they are out-competed and eliminated
  - strains with a minimum temperature tolerance above human body temperature so that they will not multiply in the human body
- use of strains that contain 'suicide genes' which are activated if conditions move outside certain pH or temperature limits
- use of containment mechanisms — for example, highly efficient air filters along with regular monitoring of the atmosphere in purpose-built laboratories

There are also **ethical concerns** over tampering with DNA of different species in ways that could never happen in nature. Some people are sceptical of the ownership of this powerful technology by a handful of multinational corporations that may be more interested in profits than in the long-term welfare of humans and the environment. There are also ethical concerns surrounding the use of genetic screening, i.e. the testing for genetic disorders in parents and embryos. Germ-line gene therapy — the transfer of genes into gametes to correct a genetic disorder — is a particularly contentious issue and raises the possibility of engineering 'designer babies'.

Table 6 Examples of the benefits and risks associated with the use gene technology

	Potential benefits: arguments for the use of gene technology	**Potential risks/concerns: arguments against the use of gene technology**
Genetically engineered micro-organisms (GEMs) developed to produce protein	More economic and wider production of medically important proteins, e.g. insulin	Some of the microorganisms (e.g. *E. coli*) live normally in the human gut; GEMs could escape from the laboratory and create a new stain of 'superbug'
Genetically modified (GM) plants including GM crops	Cheaper food for richer countries Possible reduction in the use of pesticides Reduction of food shortages in poorer countries	Risk of 'genetic pollution' with the spread of new genes from the modified crop to wild species, e.g. the formation of 'super weeds' Ecological concern that genetically modified plants may out-compete wild plants Concerns about allergic reactions
Genetically modified animals for food	Increased productivity of animals such as fish and cattle by transferring the gene for growth hormone into their genome	Concern that foreign protein, produced by transferred genes, may act as antigens (allergens) and increase the likelihood of allergies
Gene therapy	Effective treatment of genetic diseases (e.g. cystic fibrosis), relieving suffering and increasing life expectancy	Introducing genes into the human genome may disrupt the functioning of other genes, as in the appearance of leukaemia in patients treated for severe combined immunodeficiency (SCID)
Human genome research	Facilitates biomedical research	Concern that information from genome research might be used to produce 'designer babies' (e.g. for 'looks' and high IQ) Concern that an individual's genomic information (e.g. regarding susceptibility to heart disease) might become available to insurance companies
Genetic screening	People will have a better understanding of the risk of passing on a genetic disorder The fetus may be tested for the disorder before birth (called prenatal diagnostic testing)	Increased risk of stress resulting from the knowledge of being a carrier or of developing a disorder later in life (e.g. Huntington's disease) Termination of a pregnancy may not be acceptable
Gene knockout technology	Better understanding of how genes function; these genes might be implicated in a genetic disorder or might mutate to cause cancer.	Large numbers of mice are used in biomedical research, many of which may be in pain; there is the view that animals have rights and that it is unacceptable to use them in this way

With so many views, there is a need for the government to make decisions, i.e. there is a need for **legislation**. In the UK, the use of gene technologies is regulated strictly and research in the area of germ-line gene therapy in humans is banned.

Tip You may expect to find questions that raise issues of an ethical nature. This is to emphasise that good scientific practice should consider not only what we 'can do' but also whether we 'should do' it. You are expected to appreciate and make informed comments on such aspects as: the ethical implications of the way research is carried out and the way in which society uses science to help in decision making. In any discussion on such issues you should be able to present a rational and balanced account, with arguments both for and against. You should find the website at **www.beep.ac.uk** helpful in developing your understanding.